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Effects of Immediate Postexercise Carbohydrate Ingestion With and Without Protein on Neutrophil Degranulation

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The purpose of the study was to determine the effects of carbohydrate (CHO) intake, with and without protein (PRO), immediately after prolonged strenuous exercise on circulating bacterially stimulated neutrophil degranulation. Twelve male runners completed 3 feeding interventions, 1 week apart, in randomized order after 2 hr of running at 75% VO_{2max}. The feeding interventions included a placebo solution, a CHO solution equal to 1.2 g CHO˜/kg body mass (BM), and a CHO-PRO solution equal to 1.2 g CHO/kg BM and 0.4 g PRO/kg BM (CHO+PRO) immediately postexercise. All solutions were flavor and water-volume equivalent (12 ml/kg BM). Circulating leukocyte counts, bacterially stimulated neutrophil degranulation, plasma insulin, and cortisol were determined from blood samples collected preexercise, immediately postexercise, and every 30 min until 180 min postexercise. The immediate postexercise circulating leukocytosis, neutrophilia, and lymphocytosis ($p < .01$ vs. preexercise) and the delayed lymphopenia (90 min postexercise, $p < .05$ vs. preexercise) were similar on all trials. Bacterially stimulated neutrophil degranulation decreased during recovery in control (23% at 180 min, $p < .01$ vs. preexercise) but remained above preexercise levels with CHO and CHO+PRO. In conclusion, CHO ingestion, with or without PRO, immediately after prolonged strenuous exercise prevented the decrease in bacterially stimulated neutrophil degranulation during recovery.

Keywords: nutrition, recovery, running, immune, endurance

The consumption of carbohydrate (CHO) and protein (PRO) immediately after prolonged strenuous exercise is recommended and commonly practiced by endurance athletes in an attempt to replenish glycogen stores, promote PRO resynthesis and tissue repair, and thus maximize the adaptation to training (American Dietetic Association et al., 2009; Hawley, Burke, Phillips, & Spriet, 2011). This dietary strategy may also play a role in maintaining immune function after prolonged strenuous exercise (Costa et al., 2009).

We recently demonstrated that consuming a CHO and PRO solution (1.2 g CHO/kg body mass [BM] and 0.4 g PRO/kg BM) immediately after prolonged strenuous exercise prevented the decrease in bacterially stimulated neutrophil degranulation that was observed with control (–24%) and with 1-hr-delayed CHO and PRO ingestion (–31%; Costa et al., 2009). Nevertheless, it remains unknown whether the addition of PRO to a CHO recovery drink is necessary to avoid the decrease in bacterially stimulated neutrophil degranulation after prolonged strenuous exercise, or whether CHO alone is sufficient. We speculated, in a previous study, that insulin-stimulating properties of CHO and PRO might have contributed to the maintenance of bacterially stimulated neutrophil degranulation; insulin has been shown to increase bactericidal capacity in healthy individuals, possibly via a priming effect (Stegenga et al., 2008; Walrand, Guillet, Boirie, & Vasson, 2006). Considering the role of neutrophil degranulation in first-line defense against foreign pathogens and removal of damaged tissue (e.g., after prolonged strenuous exercise), preventing the decrease in neutrophil degranulation using recovery feeding strategies may influence infection incidence and training adaptations (Costa et al., 2009; Peake, 2002). If insulin does play a role in maintaining neutrophil degranulation during recovery from prolonged strenuous exercise, the addition of PRO to a CHO beverage may be more beneficial than CHO alone. In line with this contention, studies have reported greater insulin responses with ingestion of CHO and PRO, compared with CHO alone, after glycogen-depleting exercise protocols (van Loon, Kruijshoop, Verhagen, Saris, & Wagenmakers, 2000).

A practical limitation of the studies demonstrating immune-modulating effects of CHO during prolonged strenuous exercise (Bishop, Gleeson, Nicholas, & Ali, 2002; Nehlsen-Cannarella et al., 1997; Nieman et al., 1998) and, more recently, in the recovery period after exercise (Costa et al., 2009) is that these studies were performed in fasted participants; it is not a common practice for athletes to train or compete fasted. Indeed,

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one recent study demonstrated that when endurance athletes were provided with a substantial CHO meal (2.1 g CHO/kg BM) 2 hr before prolonged strenuous exercise, as is commonly practiced by endurance athletes, CHO ingestion during exercise had little effect on circulating cytokine responses (Cox, Pyne, Cox, Callister, & Gleeson, 2008). The influence of recovery feeding strategies on bacterially stimulated neutrophil degranulation in endurance athletes who consume a typical preexercise meal remains unknown.

With this in mind, the aim of the current study was to determine the influence of CHO feeding immediately after prolonged strenuous exercise, with and without the addition of PRO, on bacterially stimulated neutrophil degranulation in trained runners. We hypothesized that such feeding would attenuate changes in bacterially stimulated neutrophil degranulation during recovery.

Methods

Participants

Twelve healthy competitive endurance runners (*M* ± *SD:* age 29 ± 7 years, nude BM 77 ± 8 kg, height 1.78 ± 0.05 m, % body fat 15 ± 4, VO_{2max} 63 ± 5 ml · kg^{-1} · min^{-1}) volunteered to participate in the study. All participants were club-level road and fell runners with an average of 10 ± 7 years of competition experience. All gave written informed consent before the study, which received approval from the local ethics committee. Participants reported no symptoms of infection or illness, and they took no medications in the 12 weeks before or during the study. They were asked to complete a health and training log during their time adhering to experimental procedures. They were also asked to refrain from alcohol and caffeine for 72 hr, and exercise for 24 hr, before preliminary testing sessions and each experimental trial. All volunteers were nonsmokers.

Preliminary Measurements

One week before the first experimental trial, the participants were asked to report to the laboratory and dress in athletic shorts and T-shirt, where height (stadiometer, Bodycare Ltd., Warwickshire, UK) and nude BM (STW-150KE, Hampel Electronics, Zhonghe, Taiwan) were recorded. Body composition was determined by whole-body dual-energy X-ray absorptiometry (Hologic QDR1500, software version 5.72, Bedford, MA, USA). Maximal oxygen uptake (VO_{2max}; Cortex Metalyser 3B, Biophysik, Leipzig, Germany) was estimated by means of a continuous incremental exercise test to volitional exhaustion on a motorized treadmill (h/p/cosmos Mercury 4.0, Nussdorf-Traunstein, Germany). The exercise test began with a treadmill speed of 8 km/hr and 1% inclination. Speed was increased by 2 km/hr every 3 min until reaching 16 km/hr, when inclination was increased by 2.5% every 3 min until the participant reached volitional exhaustion. Criteria for attaining VO_{2max} included reaching volitional exhaustion (rating of perceived exertion at 20; Borg, 1982), a heart rate within 10 beats/min of age-predicted maximal heart rate, and a respiratory-exchange ratio ≥1.15 (Bird & Davison, 1997). Participants performed a further 15-min treadmill familiarization during which the treadmill speed at 75% VO_{2max} was extrapolated and verified (11.5 ± 1.2 km/hr and 1% gradient).

Experimental Trials

To control dietary intake, before each experimental trial a 24-hr diet was provided that catered to the participants' estimated energy requirements (2,963 [12.4 MJ] ± 193 kcal; Harris & Benedict, 1919) and provided 60% CHO, 23% fat, 17% PRO, and 35 ml/kg BM of water (2,698 ± 285 ml; Todorovic & Micklewright, 2004). On three occasions separated by 1 week, participants reported to the laboratory at 7 a.m. after an overnight fast, where a standard breakfast (526 [2.2 MJ] kcal; 118 g CHO, 9 g PRO, 2 g fat) with 400 ml fluid was provided 2 hr before the onset of exercise. They were asked to empty their bladder and bowel before preexercise nude BM measurements. Urine specific gravity, determined by a handheld refractometer (Atago Uricon-Ne, NSG Precision Cells, New York, NY) from a midflow urine sample collected into 30-ml universal tubes, was <1.020 g/L in all participants on arrival at the laboratory for each trial, indicating euhydration (Armstrong et al., 1994). After preexercise measurements and sample collection, participants ran on a motorized treadmill for 2 hr at the previously determined treadmill speed that elicited 75% VO_{2max}, dressed in athletic shorts and T-shirt. The 2-hr exercise bout was performed in ambient conditions of 20 °C and 59% relative humidity with two fans running at a speed of 2.3 m/s placed 1 m from the treadmill (VelociCal, TSI, St. Paul, MN). Heart rate and rating of perceived exertion (Borg, 1982) were recorded every 10 min during exercise. Participants were given water at a rate of 3 ml · kg^{-1} BM · hr^{-1} (231 ± 24 ml/hr) during exercise. This water was provided as one bolus in the first hour and one bolus in the second hour.

After the postexercise blood- and saliva-sample collections, nude BM was recorded, and in randomized order participants performed one of three recovery interventions involving the same fluid volume (12 ml/kg BM), flavor (strawberry), and timing (immediately after exercise) of a recovery solution. The recovery solution consisted of either an artificially sweetened placebo for the control group (CON: 0 g of CHO and PRO; Science in Sport, Blackburn, UK), a CHO drink made to a 10% CHO solution that provided 1.2 g CHO/kg BM (Science in Sport), or a CHO and PRO drink made to a 10% CHO solution that provided 1.2 g CHO/kg BM and 0.4 g PRO/kg BM (Science in Sport). Participants received a further placebo bolus 1 hr postexercise in all trials. They remained in the laboratory for the entire 180-min recovery period during which samples were collected.

Sample Collection and Analysis

Whole-blood samples were collected by venipuncture from an antecubital vein preexercise, immediately postexercise, and at 30-min intervals postexercise for 180 min into two K_3EDTA tubes (4 ml, 1.6 mg/ml of ethylenediaminetetraacetic acid; Becton Dickinson, Oxford, UK) and two lithium heparin tubes (4 ml, 1.5 IU/ml of heparin; Becton Dickinson). For each sample taken, one K_3EDTA Vacutainer tube was stored at room temperature before hematological analysis within 6 hr of collection. Hematological analysis, which included hemoglobin concentration and total and differential circulating leukocyte counts, was performed using an automated cell counter (Gen-S, Beckman Coulter, High Wycombe, UK) at the local hospital (Haematology Department, Ysbyty Gwynedd, UK). Hematocrit was determined by capillary method in triplicate using lithium heparin blood samples and a microhematocrit reader (Hawksley & Sons Ltd., Lancing, UK). All blood-borne immune and hormonal indices measured were corrected for changes in plasma volume (Dill & Costill, 1974). An additional 1 ml of lithium heparin blood was used to determine bacterially stimulated neutrophil elastase release. The remaining lithium heparin and K_3EDTA samples were centrifuged at 1,500 *g* for 10 min at 4 °C within 15 min of sample collection. Plasma samples were aspirated into Eppendorf tubes and stored at –80 °C for further analysis.

A 1-ml volume of lithium heparin blood was added to 50 µl of 10 mg/ml bacterial stimulant (Sigma, Poole, UK) within 5 min of collection and gently vortex-mixed as described previously (Laing, Blackwell, Gwynne, Walters, & Walsh, 2005; Robson, Blannin, Walsh, Castell, & Gleeson, 1999). Samples were immediately placed in a water bath at 37 °C for 60 min and further mixed by gentle inversion at 30 min. After 60 min, they were centrifuged at 5,000 *g* for 2 min. Supernatant was then aspirated into Eppendorf tubes and stored at –80 °C for further analysis. Plasma elastase concentration and elastase release per neutrophil, which are markers of neutrophil degranulation activity, as reported previously (Laing et al., 2005; Robson et al., 1999), were determined using enzyme-linked immunosorbent assay (ELISA; Biovendor Laboratory Medicine, Modrice, Czech Republic) in unstimulated and bacterially stimulated lithium heparin plasma. All samples used to determine plasma elastase concentration were run on the same day, with standards and controls for each plate. The intra-assay coefficient of variation for plasma elastase concentration was 2.1%.

Plasma glucose was determined in heparinized plasma using a spectrophotometric kit (Randox, County Antrim, UK). Aliquots of heparinized plasma were also used to determine plasma insulin (DRG Diagnostics, Marburg, Germany) and cortisol concentration using ELISA (DRG Diagnostics). All insulin and cortisol samples analyzed were run on the same day, with standards and controls on each plate. The intra-assay coefficients of variation for plasma glucose, insulin, and cortisol were 3.4%, 3.8%, and 3.5%, respectively.

Statistical Analysis

The data were examined using two-way repeated-measures ANOVA, except BM loss, which was assessed using a one-way repeated-measures ANOVA. Assumptions of homogeneity and sphericity were checked, and, where appropriate, adjustments to the degrees of freedom were made using Greenhouse-Geisser correction. Significant main effects were analyzed using post hoc Tukey's honestly significant difference test or Bonferroni-corrected *t* tests where appropriate. Data in text and tables are presented as $M \pm SD$. For clarity, data in figures are presented as $M \pm SEM$. The required sample size was estimated to be 4–12 participants (www.dssresearch.com/toolkit/sscalc) using previous data examining the effects of exercise stress on selected immune indices (Bishop et al., 2002; Costa et al., 2009; Laing et al., 2005; Nehlsen-Cannarella et al., 1997). Alpha and beta levels were set at .05 and .8, respectively, both of which are standard estimates (Jones, Carley, & Harrison, 2003). The acceptance level of significance was set at $p < .05$.

Results

There was no significant interaction for heart rate (exercise mean: 144 ± 45 beats/min) and rating of perceived exertion (exercise mean: 11 ± 4), and no significant difference in BM loss was observed between trials (mean of trials: 2.4% ± 0.4%). There was a significant main effect of time for plasma volume change ($p < .01$). A significant decrease (–5.3% ± 5.2%) in plasma volume was observed postexercise compared with preexercise ($p < .01$).

Circulating Leukocyte, Neutrophil, and Lymphocyte Counts and Neutrophil:Lymphocyte Ratio

There were no Trial × Time interactions for circulating leukocyte, neutrophil, or lymphocyte counts or the neutrophil:lymphocyte ratio. However, a significant main effect of time was observed for circulating leukocyte, neutrophil, and lymphocyte counts and the neutrophil:lymphocyte ratio ($p < .01$). A significant circulating leukocytosis ($p < .01$; Table 1) and neutrophilia ($p < .01$; Figure 1[A]) were observed after exercise and remained significantly elevated throughout the recovery period measured. Circulating lymphocyte counts significantly increased immediately postexercise ($p < .01$), and a significant lymphopenia was observed at 90 min and 120 min postexercise ($p < .05$; Table 1). Compared with preexercise values (CON 1.9 ± 0.8, CHO 1.5 ± 0.4, CHO+PRO 1.5 ± 0.3), the neutrophil:lymphocyte ratio significantly increased by 30 min postexercise ($p < 0.01$) and continued to progressively increase throughout recovery, peaking at 120 min postexercise (CON 7.4 ± 2.6, CHO 6.1 ± 2.0, CHO+PRO 7.4 ± 3.6).

Table 1 Responses of Circulating Leukocyte and Lymphocyte Count ($\times 10^9$/L) to Placebo (CON), Carbohydrate Alone (CHO), and CHO With Protein (CHO+PRO) Feeding Immediately After 2 hr of Running at 75% VO_{2max}, $M \pm SD$ (N = 12)

			Recovery Period (min)					
	Pre	**Post**	**30**	**60**	**90**	**120**	**150**	**180**
Leukocyte		††	††	††	††	††	††	††
CON	5.4 ± 0.8	9.8 ± 2.2	9.3 ± 2.2	10.1 ± 1.7	11.0 ± 1.4	11.3 ± 1.8	11.5 ± 1.6	11.2 ± 1.6
CHO	5.1 ± 0.6	10.2 ± 2.0	9.2 ± 2.0	9.5 ± 1.8	10.4 ± 1.9	10.9 ± 1.9	10.8 ± 1.7	10.7 ± 1.7
CHO+PRO	5.0 ± 0.9	9.7 ± 1.6	9.2 ± 1.6	9.8 ± 1.3	10.7 ± 1.7	11.3 ± 1.6	11.3 ± 1.7	11.2 ± 1.6
Lymphocyte		††			†	†		
CON	1.8 ± 0.4	2.8 ± 0.8	1.7 ± 0.4	1.5 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	1.5 ± 0.3	1.6 ± 0.3
CHO	1.8 ± 0.3	2.8 ± 0.6	1.6 ± 0.3	1.5 ± 0.3	1.4 ± 0.4	1.5 ± 0.3	1.6 ± 0.3	1.7 ± 0.3
CHO+PRO	1.8 ± 0.3	2.8 ± 0.7	1.7 ± 0.4	1.4 ± 0.4	1.4 ± 0.3	1.4 ± 0.3	1.5 ± 0.4	1.8 ± 0.5

†$p < .05$ and ††$p < .01$ vs. pre, main effect of time.

Neutrophil Degranulation

A significant main effect of time only ($p < .01$) was observed for unstimulated plasma elastase concentration. Compared with preexercise values (CON 54 ± 33 ng/ml, CHO 46 ± 9 ng/ml, CHO+PRO 49 ± 31 ng/ml; $p < .01$), unstimulated plasma elastase concentration significantly increased immediately postexercise (CON 219 ± 88 ng/ml, CHO 178 ± 51 ng/ml, CHO+PRO 229 ± 104 ng/ml).

Significant Trial × Time interactions were observed for bacterially stimulated elastase concentration ($p < .01$; Figure 1[B]) and bacterially stimulated elastase release per neutrophil ($p < .01$; Figure 1[C]). In all trials bacterially stimulated elastase concentration significantly increased postexercise ($p < .01$) and remained elevated throughout the recovery period measured. Compared with CON, bacterially stimulated elastase concentration was significantly higher 60 min postexercise onward with CHO ($p < .01$) and 30 min postexercise onward with CHO+PRO ($p < .01$). Bacterially stimulated neutrophil degranulation (bacterially stimulated elastase release per neutrophil) significantly decreased at 60 min postexercise in CON (–23%) and remained significantly lower throughout the recovery period (–23% at 180 min postexercise; $p < .01$ vs. preexercise). CHO and CHO+PRO prevented the decrease in bacterially stimulated neutrophil degranulation observed in CON. A significant increase in bacterially stimulated neutrophil degranulation was observed in CHO 90 min (22%) and 180 min (28%) postexercise ($p < .01$ vs. preexercise). Compared with CON, neutrophil degranulation was significantly higher 60 min postexercise onward with CHO ($p < .01$) and 30 min postexercise onward with CHO+PRO ($p < .01$).

Plasma Glucose, Insulin, and Cortisol Concentration

A significant Trial × Time interaction ($p < .01$) was observed for plasma glucose and insulin concentrations (Figure 2[A] and [B]), and a significant main effect of time was observed for plasma cortisol concentration ($p < .01$; Figure 2[C]). Plasma glucose concentration was unaffected by 2 hr of running at 75% VO_{2max} in all trials. Significant increases in plasma glucose concentration were observed after CHO and CHO+PRO bolus ingestion only ($p < .01$ vs. postexercise), which remained elevated until 150 min postexercise ($p < .05$ and $p < .01$ vs. postexercise, respectively). Compared with CON, plasma glucose concentration was significantly higher with CHO and CHO+PRO at 30 min until 150 min postexercise ($p < .01$).

Similarly, significant increases in plasma insulin concentration were observed after CHO and CHO+PRO bolus ingestion only ($p < .01$ vs. postexercise; Figure 2B), which remained elevated until 120 min postexercise in CHO ($p < .01$) and 150 min postexercise in CHO+PRO ($p < .01$). Plasma insulin concentration was significantly higher than CON at 30 min until 120 min postexercise with CHO ($p < .01$) and 30 min until 150 min postexercise with CHO+PRO ($p < .01$). In addition, plasma insulin concentration was significantly higher with CHO+PRO than with CHO at 60 min and 90 min postexercise ($p < .01$). Plasma cortisol concentration increased immediately postexercise, peaking at 30 min postexercise (59%; $p < .01$ vs. preexercise), and returned to preexercise levels by 120 min postexercise.

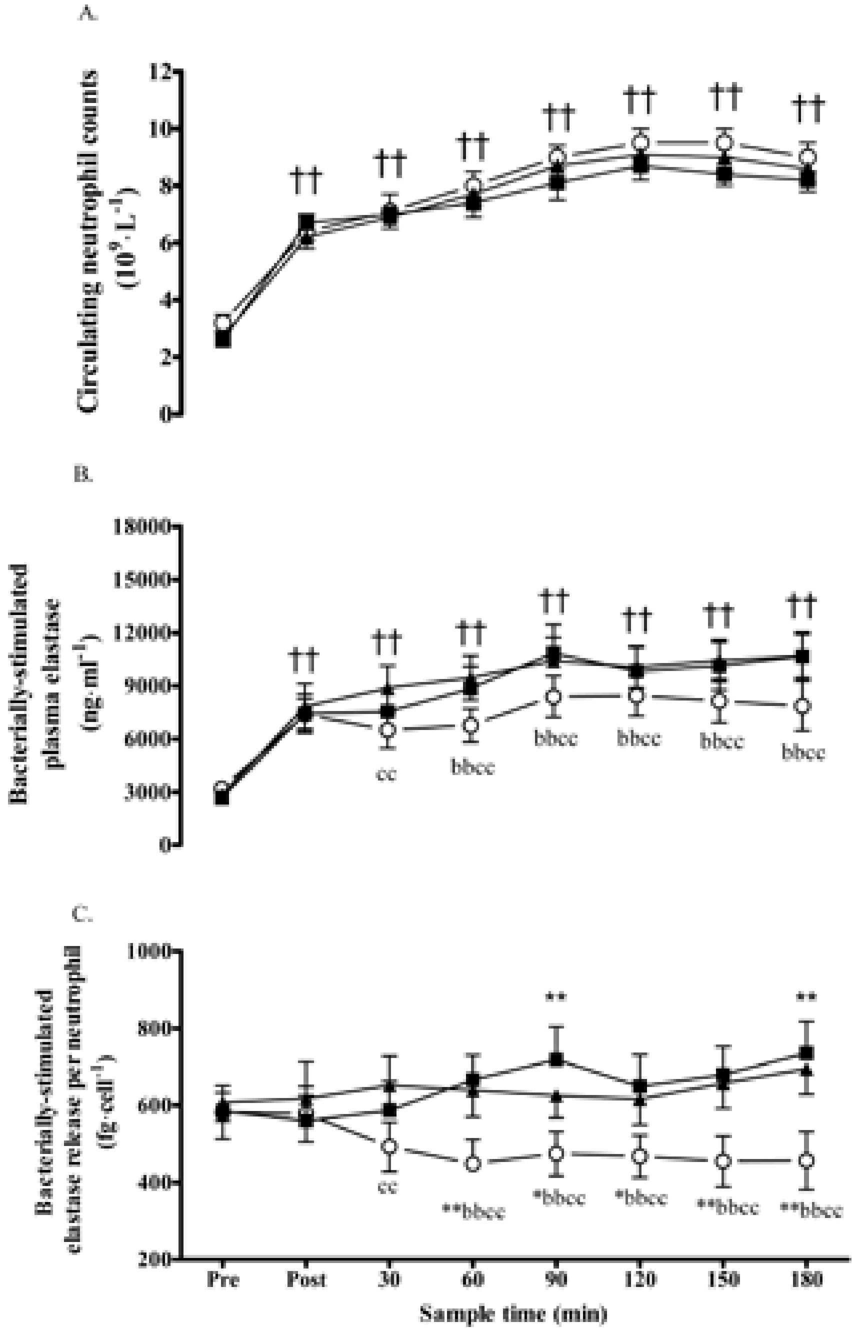

Figure 1 — Responses of (A) circulating neutrophil count, (B) bacterially stimulated plasma elastase concentration, and (C) bacterially stimulated elastase release per neutrophil to placebo (CON), carbohydrate alone (CHO), and CHO with protein (CHO+PRO) feeding immediately after 2 hr of running at 75% VO_{2max}, $M \pm SEM$ (N= 12). Closed square = CHO; closed triangle = CHO+PRO; open circle = CON. Main effect of time ††$p < .01$ vs. pre; *$p < .05$ and **$p < .01$ vs. pre within trial; $^{bb}p < .01$ vs. CHO; $^{cc}p < .01$ vs. CHO+PRO at same time point.

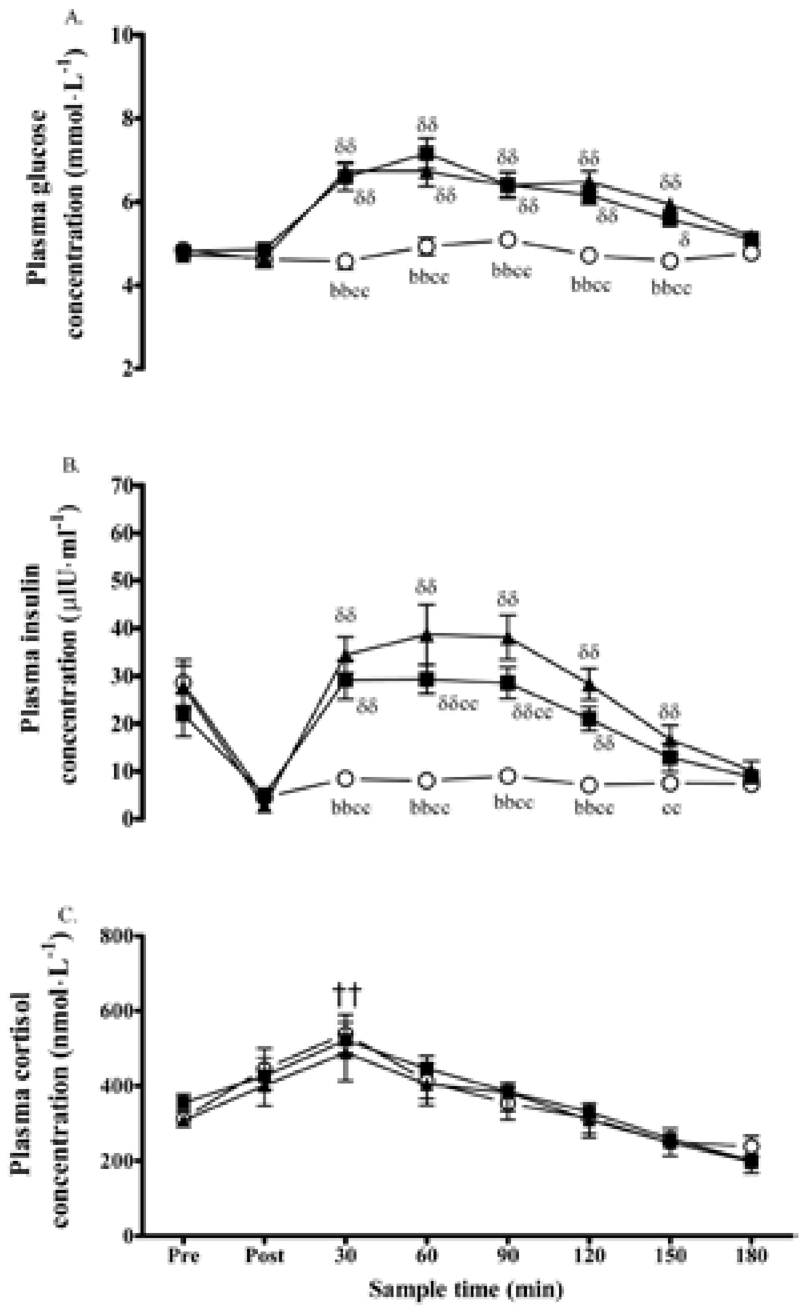

Figure 2 — Responses of (A) plasma glucose, (B) insulin, and (C) cortisol to placebo (CON), carbohydrate alone (CHO), and CHO with protein (CHO+PRO) feeding immediately after 2 hr of running at 75% VO_{2max}, $M \pm SEM$ ($N = 12$). Closed square = CHO; closed triangle = CHO+PRO; open circle = CON. Main effect of time $^{\dagger\dagger}p < .01$ vs. pre; $^{\delta}p < .05$ and $^{\delta\delta}p < .01$ vs. postexercise within trial; $^{bb}p < .01$ vs. CHO; $^{cc}p < .01$ vs. CHO+PRO at same time point.

Discussion

The aim of the current study was to determine the influence of CHO feeding immediately after prolonged strenuous exercise either with or without the addition of PRO on bacterially stimulated neutrophil degranulation in trained runners. In line with our hypothesis, we showed that providing CHO alone (1.2 g CHO/kg BM) immediately after prolonged strenuous exercise was sufficient to prevent the decrease in bacterially stimulated neutrophil degranulation, but the addition of PRO (0.4 g PRO/kg BM) did not alter this response. A practical strength of this study is that we investigated the influence of recovery feeding on immune indices after prolonged strenuous exercise preceded by a typical preexercise meal, as is often practiced by endurance athletes, rather than after an overnight fast, as was performed previously (Costa et al., 2009).

The current findings extend previous ones because they indicate that CHO, and not PRO, was the likely key nutrient in a recovery drink containing both CHO and PRO that prevented the decrease in bacterially stimulated neutrophil degranulation after prolonged strenuous exercise (Costa et al., 2009). Decreases in circulating neutrophil function have frequently been reported during recovery from prolonged exercise (Costa et al., 2009; Robson et al., 1999). Even though the clinical significance of a decrease in circulating neutrophil function for endurance athletes is still unclear, impaired neutrophil function has been associated with increased bacterial and fungal infection incidence, albeit in clinical populations (Alba-Loureiro et al., 2007; Ellis et al., 1988). Whether the frequency or severity of bacterial and fungal infections is increased by endurance training remains a topic of debate (Walsh et al., 2011). Nevertheless, suboptimal neutrophil function may contribute to illness and infection risk in endurance athletes during recovery from exercise stress (Peake, 2002). From a practical standpoint, the findings of the current study together with previous findings (Costa et al., 2009) indicate that the immune systems of endurance athletes who perform prolonged strenuous exercise in the morning with no CHO ingestion during exercise may benefit from CHO ingestion (≥1.2 g CHO/kg BM) immediately after exercise, regardless of whether the exercise is performed in the fed or overnight-fasted state. From a critical standpoint, it should be noted that these findings only relate to one aspect of innate immune function, and further studies should investigate the effect of recovery feeding on other aspects of immune function (e.g., lymphocyte proliferation and in vivo immune responses).

Exercise immunologists have proposed various blood-borne mechanisms (e.g., decreased plasma glucose concentration, increases in plasma stress hormones and IL-6) to explain the decrease in bacterially stimulated neutrophil degranulation after prolonged strenuous exercise (Bishop et al., 2002; Robson et al., 1999). The current findings and other more recent findings do not fully support these proposed blood-borne mediators (Costa et al., 2009; Laing et al., 2008). For example, although neutrophils rely heavily on glucose for energy supply (Furukawa et al., 2000), a direct effect of decreased glucose availability on neutrophil degranulation is unlikely because we did not observe a decrease in plasma glucose concentration after exercise (mean of trials: pre- 4.8 ± 0.5 vs. post- 4.7 ± 0.6 mmol/L), and plasma glucose concentration remained within the normal range during recovery in CON (4.8 ± 0.6 mmol/L) when bacterially stimulated neutrophil degranulation decreased. In addition, similar decreases in bacterially stimulated neutrophil degranulation have been observed after prolonged strenuous exercise both under control conditions and when experimental manipulations blunted stress hormones and IL-6 responses to exercise (by exercising with a thermal clamp; Laing et al., 2008). Nevertheless, it is quite conceivable that the increase in plasma cortisol concentration after prolonged exercise in the current study may have contributed to the decrease in bacterially stimulated neutrophil degranulation in CON, whereas another stimulatory mechanism may have been responsible for the maintained bacterially stimulated neutrophil degranulation with CHO and CHO+PRO (e.g., insulin response; Costa et al., 2009).

The current study shows that providing CHO early during recovery can counter the decrease in bacterially stimulated neutrophil degranulation after prolonged strenuous exercise. A direct stimulatory effect of increased glucose availability on bacterially stimulated neutrophil degranulation seems unlikely because hyperglycemia has been shown to decrease neutrophil degranulation, albeit in diabetes mellitus patients with poor glycemic control (Alba-Loureiro et al., 2007; Stegenga et al., 2008). Recently, a potential role for insulin in modulating neutrophil function has been proposed (Stegenga et al., 2008; Walrand et al., 2006). Studies using a hyperinsulinemic-euglycemic clamp have shown that raised circulating insulin increased neutrophil chemotaxis, phagocytosis, and bactericidal capacity in healthy individuals, possibly via a priming effect of insulin (Walrand et al., 2006). It is conceivable that the sustained increase in plasma insulin concentration during recovery with CHO and CHO+PRO may have prevented the decrease in bacterially stimulated neutrophil degranulation observed in CON. Although the addition of PRO to the CHO solution led to a greater circulating insulin response (60 and 90 min postexercise with CHO+PRO compared with CHO), it appears not to provide further beneficial effect on bacterially stimulated neutrophil degranulation during recovery from prolonged strenuous exercise. It is quite conceivable that there is a threshold whereby insulin promotes its effects on neutrophil function, possibly via a degree of insulin-receptor saturation (Walrand, Guillet, Boirie, & Vasson, 2004; Walrand et al., 2006). Future studies could investigate the putative role of insulin in maintaining neutrophil function after prolonged strenuous exercise by, for example, adding insulin to postexercise blood samples in vitro.

In conclusion, these results show that consuming CHO alone (1.2 g CHO/kg BM) immediately after prolonged strenuous exercise prevented the decrease in bacterially stimulated neutrophil degranulation, but the addition of PRO (0.4 g PRO/kg BM) did not alter this response. Future studies should determine whether insulin plays a pivotal role in maintaining bacterially stimulated neutrophil degranulation with CHO feeding immediately after prolonged strenuous exercise.

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